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USE OF "LYSIS BUFFER" IN DNA ISOLATION AND ITS IMPLICATION FOR MUSEUM COLLECTIONS

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ADVANTAGES OF USING "LYSIS BUFFER"

Many molecular techniques require relatively large quantities of high molecular weight DNA and several protocols for collection are well established if there are unlimited numbers of samples or if all procedures can be performed under the stable conditions afforded in a laboratory. Unfortunately, the collection of DNA for many species, populations, or samples does not afford such stable conditions. Conservation genetic studies of rare and endangered species often force opportunistic collections which involve road or other accidental kills, exploitation of dead animals at zoos, collection under extreme field conditions, etc. The method described below, which is a modification of a procedure described by Longmire et al. (1991), has several advantages. First, relatively small amounts of tissue routinely provide large quantities of DNA, including both nuclear and mitochondrial components. Second, this method consistently yields high molecular weight DNA that can be used for all molecular techniques including cosmid library construction. Third, nuclear DNA can be obtained from essentially any tissue that contains nucleated cells. This would include, but not be limited to, nucleated red blood cells of lower vertebrates and birds, muscle, heart, liver, kidney, testes, embryonic tissue from placentas or eggs, pulp of growing feathers, skin biopsies, and hair follicles. Fourth, samples collected in lysis buffer do not require refrigeration. Fifth, field procedures are relatively simple and inexpensive. Sixth, scissors and forceps are the only equipment that need to be cleaned between samples to protect against cross contamination. Finally, a small kit and limited equipment allows one to be prepared for opportunistic situations.

At the heart of this procedure is a buffer solution that dissolves and neutralizes the cellular components. When tissue is placed in this buffer, the DNA is freed from cellular membranes and becomes soluble. When the samples reach the laboratory, Proteinase K is used to further break down the cellular proteins. This is followed by phenol extractions and dialysis. At this point, the DNA is ready for molecular biological procedures.

DISADVANTAGES OF USING "LYSIS BUFFER"

The major problem associated with using lysis buffer is the loss of proteins which have a broad number of potential uses including starch-gel electrophoresis of allozymes. Additionally, proteins are useful in addressing health related issues such as the detection of anti-

bodies and some ecotoxicological molecules. It is also likely that RNA is compromised to some extent. How this might effect the construction of cDNA libraries or reverse transcriptase polymerase chain reactions has not been tested. Cell structures that are available from frozen samples also are lost. For these reasons, where possible, we save both frozen and lysis buffer samples from each specimen.

MUSEUM USAGE

The Museum of Texas Tech University is committed to the curation and care of frozen tissues to provide samples for research in conservation genetics, systematics, etc. (Baker, 1994). We regularly use this method to isolate DNA from samples from our frozen tissue collection because we obtain greater yields of high molecular weight DNA compared to other methods tested in our laboratory. Additionally, when we receive loan requests for DNA samples from the frozen tissue collection, these are sent out in lysis buffer. If the sample tubes are adequately protected, no special handling is required. Using this buffer system eliminated several problems including those associated with dry ice shipments.

PROTOCOL FOR DNA ISOLATION

Collection of the sample for isolation.-- For blood containing nucleated red blood cells, 0.5 ml of blood yields about 5 mg of genomic DNA. Add 0.25- 0.5 ml freshly drawn blood to a 15 ml sterile polypropylene tube (Corning or Falcon brand) containing 5 ml of "Lysis Buffer" (described in Formula 1). Do not add more than 0.5 ml since it has been observed from past experiments that too much blood can overload the buffer and the DNA can become degraded. After blood is added, invert the tube several times. The solution should become viscous. Samples can be stored at ambient temperatures, but should be protected from extreme heat. In the case of tissues (such as liver, brain, spleen, and muscle), no more than 0.3-0.5 grams are added to 5 ml of lysis buffer. Invert the tube several times to increase the exposure of the tissue to the buffer. For tissue such as liver, brain, and spleen, it does not appear necessary to dice the tissue into quantities smaller than 0.5 grams. For organs that are protected by well developed layers of connective tissue (such as testes), it is necessary to dice the tissue into smaller pieces. Brain tissue seems to yield the highest quality DNA in cases where the animal has already begun to decompose. Blood and tissue DNA isolation procedures not only produce high molecular weight DNA, they also have the advantage that no refrigeration is required after the cells are dissolved in the lysis buffer. Samples are usually stored in a cool place, out of direct sunlight. Samples that are mailed should be in a styrofoam cooler or otherwise insulated, if possible. Samples can be stored at room temperature for several years prior to DNA isolation (Longmire et al., 1991). Samples that will be used for making recombinant libraries should not be frozen to avoid shearing of the DNA.

Because PCR can amplify small traces of DNA it is important to not cross-contaminate samples when more than one animal is being biopsied. Scissors and forceps should be meticulously cleaned between individuals. Under field conditions, we find that the use of multiple pairs of forceps and scissors results in less disruption to the processing of larger numbers of specimens. Razor blades are advantageous because they can be disposed of after a single use.

Cleaning and isolating the DNA.-- Proteinase K is added to a final concentration of 500 micrograms per ml. Place samples on a tube rotator in a 37°C incubator and rotate slowly overnight. This step is important for breaking up clumps. After overnight incubation, phenol that is saturated with TE buffer is either pre- warmed to 50°C or the extraction procedure is performed in an incubator at 37°C. Add an equal volume of phenol to each tube and rotate at a moderate speed for approximately 30 minutes. The tubes are then centrifuged at room temperature for 5 minutes at 2000 rpm to separate phases. Remove the aqueous phase, and perform a second phenol extraction. After the second extraction, the aqueous phase is placed into dialysis tubing (MWCO of 12-14,000), and dialyzed for 24 to 48 hours against three changes of 1 X TE at 4°C until the odor of phenol can no longer be detected. Final purified DNA samples may be stored at 4°C if the DNA is to be utilized within a moderate time frame (up to one year) However, if the DNA is to be stored for a longer periods or archived in a museum collection, freezing of the sample is appropriate. Equipment and chemicals used in the process can be seen in Table 1.

Table 1. List of specialized equipment for DNA isolation procedure.

DESCRIPTION	SOURCE
15 ml polypropylene tubes sterile, 50/rack, printed, threaded plug cap	Baxter, VWR,Corning
Proteinase K	Boehringer Mannheim
Phenol (crystallized or redistilled) 500g	Sigma, BRL molecular biology grade
Ethylene diaminetetraacetic acid disodium salt dihydrate ACS reagent, EDTA	Sigma
Lauryl sulfate, SDS, sodium dodecyl sulfate	Sigma
NaCl	Sigma
Trizma base	Sigma
Dialysis tubing	Fisher Scientific
Spectra/ Por 2 membranes 12,000-14,000 MWCO	
Hematology/ Chemistry Mixer	Fisher Scientific

FORMULA 1. "LYSIS BUFFER"

To make 1 liter of "Lysis Buffer" (add in numerical order):

1. 50 ml of 2 M Tris-HCL, pH 8.0
2. 200 ml of 0.5 M EDTA, pH 8.0
3. 2 ml of 5 M NaCl
4. up to 975 ml double-distilled water
5. 25 ml of 20% SDS (w/v)

LITERATURE CITED

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